Remarks

Status of the Application

Claims 1-33 were pending in the application at the time the Office Action was mailed. Claims 5-6, 15-26, and 29 were withdrawn from consideration. Claims 1-4, 7-14, 27, 28, and 30-33 were rejected. No claims were allowed. In this response, claims 1, 2, 4, 9, 12, 14, 27, and 32 have been amended, and claims 3, 5, 6, 13, 28, and 30 have been cancelled. No claims have been added. Therefore, claims 1, 2, 4, 7-12, 14, 27, and 31-33 as amended remain pending and before the examiner for consideration.

Objections

Claims 1, 3, 9, 13, 27, 28, and 30 were objected to for recitation of "LuxA protein," the Office Action alleging that "LuxA" is an abbreviation. Applicants submit that "LuxA" is not an abbreviation for a protein name, but is itself the name of the protein. For further clarification, the word "luciferase" as used in the present specification, refers to the heterodimeric enzyme that includes both LuxA and LuxB proteins.

Claim 32 was objected to for recitation of the abbreviation "IRES." Claim 32 has herewith been amended to recite "internal ribosome entry site (IRES)" instead of "IRES."

Claims 1, 2, 7, 8, 9-12, 27 and 30-33 were objected to as encompassing non-elected subject matter. These claims (or the claims from which they depend) have been amended herewith to omit recitation of the non-elected subject matter (i.e., LuxB

protein).

Withdrawal of these objections is respectfully requested.

Rejections Under 35 U.S.C. 112 – Written Description

Claims 1-3, 7-13, 27, 28, and 30-33 were rejected under the first paragraph of 35 U.S.C. 112, as failing to comply with the written description requirement. According to the examiner, these claims are directed to "a codon-optimized nucleotide sequence encoding at least one component of bacterial luciferase system LuxA protein." Applicants note for the record that independent claims 1, 9, 19, 27, and 31 filed with the Preliminary Amendment on January 26, 2005, did not recite this limitation but instead were directed to "a codon-optimized nucleotide sequence encoding at least one component of a bacterial luciferase system selected from the group consisting of a bacterial LuxA protein and a bacterial LuxB protein." Nonetheless, independent claims 1, 9, 27, and 31 have herewith been amended to recite "a codon-optimized nucleotide sequence encoding a bacterial LuxA protein" so that they no longer encompass non-elected subject matter.

To satisfy the written description requirement, a patent specification must describe the claimed invention in sufficient detail that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention. See, e.g., *Moba, B.V. v. Diamond Automation, Inc.*, 325 F.3d 1306, 1319, 66 USPQ2d 1429, 1438 (Fed. Cir. 2003) and *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d at 1563, 19 USPQ2d at 1116.

Although nearly the entire specification describes codon-optimized nucleotide sequences encoding a bacterial LuxA protein, Applicants point in particular to the Examples section, beginning on page 14 of the specification, to demonstrate that the specification describes the claimed invention in *more than* sufficient detail that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention. For example, see Example 2 entitled "Codon-optimized *luxA* and *luxB*" beginning on page 15. This example describes, among other things, how to: 1) determine codon-optimized sequences of *luxA* and *luxB* genes, 2) synthesize the codon-optimized *luxA* and *luxB* genes, 3) clone these codon-optimized genes into expression vectors, 4) transform bacterial cells with constructs containing these codon-optimized genes, 5) select bacterial clones and sequence the constructs, 6) transfect mammalian cells with these constructs, 7) select mammalian cell clones, 8) perform bioluminescence assays on these mammalian cells, 9) perform *in vitro* transcription/translation of the codon-optimized genes, and 10) compare expression of the codon-optimized genes vs. wild-type genes *in vivo*. In addition, an example of "a codon-optimized nucleotide sequence encoding a bacterial LuxA protein" is presented in the specification as SEQ ID NO:1. Furthermore, for purposes of providing adequate written description it is not required of a specification to disclose (1) examples; (2) actual reduction to practice of the invention; and (3) the known structure of a biological macromolecule. *Falko-Gunter Falkner et al. v. Stephen Inglis et al.*, Case Nos. 05-1324 (May 26, 2006) (Gajarsa J.).

Based on these teachings, a skilled artisan would easily envision a protocol for

constructing codon-optimized nucleotide sequences encoding LuxA protein. Therefore, the written description requirement in which a patent specification must describe the claimed invention in sufficient detail that one skilled in the art can reasonably conclude that the inventor had possession of the claimed invention has been met[1]. Thus, withdrawal of this rejection is respectfully requested.

Rejections Under 35 U.S.C. 112 – Enablement

Claims 1-3, 7-13, 27, 28, and 30-33 were rejected under 35 U.S.C. 112, first paragraph, because according to the Office Action, the specification, while being enabling for a codon-optimized nucleotide sequence of SEQ ID NO:1 encoding LuxA protein from *Photorhabdus luminescens*, does not reasonably provide enablement for any codon-optimized nucleotide sequence encoding any LuxA protein from any bacteria. The reasoning asserted for the enablement rejections is that the scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of codon-optimized nucleotide sequences encoding any LuxA protein having any codon substitution broadly encompassed by the claims. Applicants respectfully disagree with this assertion for the reasons described below.

With regard to meeting the enablement requirement of 35 U.S.C. 112, first paragraph, MPEP 2164.08 states that "[a]ll that is necessary is that one skilled in the art be able to practice the claimed invention, given the level of knowledge and skill in the

[1] See, e.g., *Moba, B.V. v. Diamond Automation, Inc.*, 325 F.3d 1306, 1319, 66 USPQ2d 1429, 1438 (Fed. Circ. 2003); *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d at 1563, 19 USPQ2d at 1116.

art." See *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970). Also see MPEP 2164.01 which cites *United States v. Telectronics, Inc.*, 857 F.2d 778, 785, 8 USPQ2d 1217, 1223 (Fed. Cir. 1988) ("The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation."). Applicant asserts that because of the high level of skill in the art and the state of the art at the time the application was filed, one of ordinary skill in the would not have to perform undue experimentation to make and use the invention as claimed.

In response to the examiner's assertion that the "scope of the claims is not commensurate with the enablement provided by the disclosure with regard to the extremely large number of codon-optimized nucleotide sequence encoding any LuxA protein having any codon substitution broadly encompassed by the claims," Applicants point to MPEP 2164.08 which states that "when claims are directed to any purified and isolated DNA sequence encoding a specifically named protein where the protein has a specifically identified sequence, a rejection of the claims as broader than the enabling disclosure is generally not appropriate because one skilled in the art could readily determine any one of the claimed embodiments." Further, Applicants assert that the specification does indeed teach one of skill in the art how to construct a large number of codon-optimized nucleotide sequences encoding a bacterial LuxA protein. Applicants again point to Example 2 entitled "Codon-optimized *luxA* and *luxB*" beginning on page 15, as well as all of pages 6 and 16.

Referring in particular to page 6, beginning with the third paragraph, the specification walks one step by step through the methodology of making codon-optimized nucleotide sequences encoding Lux proteins. In the third paragraph, guidance for determining a nucleotide sequence optimized for expression in mammalian cells is provided. Guidance for identifying where codon substitutions that facilitate increased expression in mammalian cells is then provided, followed by guidance for incorporating the substitutions (e.g., site-specific mutagenesis and PCR). The third paragraph further teaches that in "the examples described below, modified *lux* genes were constructed by a "recursive" PCR technique using synthesized oligonucleotides with overlapping ends as the template DNA (See, Prodromou and Pearl, Protein Engineering 5:827-829, 1992)." In the last paragraph on page 6 spanning the first paragraph of page 7, the specification provides GenBank accession numbers for each of the wild-type *lux* genes into which codon substitutions can be incorporated, and teaches that nucleic acids encoding LuxA, LuxB, LuxC, LuxD, and LuxE derived from other strains or organisms might be used so long as they can be expressed in mammalian cells to generate luminescence. The second and third paragraphs of page 8, referring to Table 1, as well as all of page 9, provide guidance for choosing preferred codons for gene expression in mammalian cells. The second paragraph of page 9 explains in sequential order the steps that are involved in creating codon-optimized nucleotide sequences encoding Lux proteins, concluding with three citations to references that teach methods of incorporating codon substititutions. Clearly, the specification does indeed teach one of skill in the art how to construct a large

number of codon-optimized nucleotide sequences encoding a bacterial LuxA protein.

Regarding the examiner's allegation that "it is not routine in the art to screen for multiple substitutions or multiple modifications, as encompassed by the instant claims, and the positions within a protein's sequence where amino acid modifications can be made with a reasonable expectation of success in obtaining the desired activity/utility are limited in any protein and the result of such modifications is unpredictable," Applicants disagree with this assertion. First, the codon substitutions are not ***amino acid*** modifications; they are ***nucleotide*** (i.e., codon) substitutions. The codon substitutions facilitate higher expression of these *lux* genes in mammalian cells but do not change the amino acid sequence of the encoded protein (see page 6, second paragraph, of the specification). Secondly, it would be routine for one of skill in the art using the considerable direction and guidance in the present specification to synthesize codon-optimized *luxA* and *luxB* genes (as taught on page 6 and on page 16, beginning with the third paragraph); the specification actually teaches how to determine codon-optimized sequences of *luxA* and *luxB* genes on pages 6 and 16 (second paragraph), how to design oligos for PCR to synthesize the genes, also on page 16, and provides a table of preferred codons for use in mammalian cells (see Table 1, page 9). MPEP 2164.06 states that "an extended period of experimentation may not be undue if the skilled artisan is given sufficient direction or guidance." *In re Colianni*, 561 F.2d 220, 224, 195 USPQ 150, 153 (CCPA 1977). Further, the fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation. *In re*

Certain Limited-Charge Cell Culture Microcarriers, 221 USPQ 1165, 1174 (Int'l Trade Comm'n 1983), *aff'd sub nom., Massachusetts Institute of Technology v. A.B. Fortia*, 774 F.2d 1104, 227 USPQ 428 (Fed. Cir. 1985).

Further regarding the test of enablement, MPEP 2164.05 states that "[t]he state of the prior art provides evidence for the degree of predictability in the art and is related to the amount of direction or guidance needed in the specification as filed to meet the enablement requirement. The state of the prior art is also related to the need for working examples in the specification." Regarding the examiner's assertion that "the disclosure is limited to the nucleotide and encoded amino acid sequence of only one codon-optimized nucleotide sequence encoding LuxA protein," MPEP 2164.02 states that "[t]he presence of only one working example should never be the sole reason for rejecting claims as being broader than the enabling disclosure, even though it is a factor to be considered along with all the other factors. To make a valid rejection, one must evaluate all the facts and evidence and state why one would not expect to be able to extrapolate that one example across the entire scope of the claims." Although only one example of a codon-optimized nucleotide sequence encoding a bacterial LuxA protein (SEQ ID NO:1) was provided in the specification, there is no reason to expect that other nucleotide sequences encoding a bacterial LuxA protein (see pages 6, 7, 16, 18) having different combinations of those codon substitutions taught in the specification (e.g., pages 8, 9, 16) would not result in an increase in LuxA protein expression in mammalian cells (compared to LuxA protein encoded by a wild-type *luxA* gene). Applicant asserts that the example in the

instant application of a codon-optimized nucleotide sequence encoding a bacterial LuxA protein, coupled with the level of skill in the art at the time the application was filed, enables one skilled in the art to make and use the claimed invention.

Because there was considerable direction and guidance in the specification as filed, a high level of skill in the art at the time the application was filed, and all of the methods needed to practice the invention were well known, it would not require undue experimentation from one of skill in the art to construct a nucleic acid having a codon-optimized nucleotide sequence encoding a bacterial LuxA protein needed to practice the claimed invention.

Claims 9, 10, and 12-14 were also rejected under 35 U.S.C. 112, first paragraph. According to the Office Action, because the specification, while being enabling for isolated host cells transformed with the recited nucleic acids, does not reasonably provide enablement for host cells within a multicellular animal which have been transformed with the recited nucleic acids. The Office Action alleges that the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

Applicants respectfully disagree with this assertion. At the time the application was filed, methods of transforming cells within complex multicellular organisms were (and still are) routine. In fact, Applicants point to U.S. patent no. 5,874,304 to Zolotukhin et al. which the examiner has used as a prior art reference in a 103 rejection. Example X in column 50 of this patent describes the expression of GFP in guinea pigs. A

number of manuals and treatises teaching how to generate transgenic animals were known and available at the time the application was filed. See, e.g., Transgenic Animal Technology: A Laboratory Notebook, 1st ed., by Carl A. Pinkert, Elsevier Publishing, Cambridge, MA, 1994; and Transgenic Animals: Generation and Use, ed. Louis-Marie Houdebine, CRC Press, Boca Raton, FL, 1997. One of skill in the art would predict that the cells described in the present specification could be successfully incorporated into a multicellular animal. Because there was considerable direction and guidance in the specification as filed and a high level of skill in the art at the time the application was filed, the specification does enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with the claims.

Withdrawal of these rejections is therefore respectfully requested.

Rejections Under 35 U.S.C. 103

Claims 1-3, 8-13, 27, 28, and 30 were rejected under 35 U.S.C.103(a) as being obvious over Szittner et al. (J Biol Chem. 1990 Sep 25; 265(27): 16581-7), Mao et al. (Zhonghua Zhong Liu Za Zhi, 2001 Sep; 23(5): 359-62) in view of Nawotka et al. (WO 03/016839) or Zolotukhin et al. (US Patent 5,874,304). Claims 31-33 were also rejected under 35 U.S.C.103(a) as being obvious over Szittner et al., Mao et al. in view of Nawotka et al. or Zolotukhin et al. and further in view of Greer et al. (Luminescence 2002 Jan-Feb; 17(1):43-74) and Lowe et al. (US Patent 6,132,983). According to the Office Action:

It would have been obvious to one of ordinary skill in the art at the time of the invention was made to combine the teachings of Szittner et al., Mao et al. and Zolotukhin et al. or Nawotka et al. to codon-optimize luciferase gene of Szittner et al. including substituting the leucine codon CTG instead of other leucine codons as disclosed by Zolotukhin and Nawotka et al. in order to optimum expression in mammalian cell and to clone the codon-optimized gene in mammalian expression vector under the regulation of promoter/enhancer as disclosed by Mao et al. to use the codon-optimized Lux system in the development a mammalian bioluminescence bioreporter system to be used in medical research and diagnostic applications.

It would have been obvious to one of ordinary skill in the art at the time of the invention was made to combine the teachings of Szittner et al., Nawotka et al., Greer et al and Lowe et al. to develop a test kit for carrying out luminescence assay to determine the gene expression as disclosed by Lowe et al. by using LuxA gene of Szittner et al. by optimizing the codon usage as taught by Nawotka et al. by using vectors having restriction sites, promoter/enhancer including IRES as well as selectable marker to determine a gene expression in a sample.

These rejections are incorrect for at least two reasons. First, the Nawotka et al. reference is not a proper 103 reference because it was published after the priority date of the present application and was filed after November 29, 2000, did not designate the U.S., and is not prior art under 35 U.S.C. §102(a) or (b). See MPEP§706.02(f)(1)I(C)(2). Second, the remaining references do not render the invention obvious within the meaning of 35 U.S.C. 103 because the combination of Szittner et al., Mao et al., Zolotukhin et al., Greer et al., and Lowe et al. fails to provide any motivation or suggestion to make the claimed invention because none disclose any reason why it would be advantageous to codon optimize *luxA*.[2]

[2] See MPEP 2143. "To establish a prima facie case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available

The Office Action states that "[o]ne of ordinary skill in the art would have been motivated to use codon-optimized LuxA gene for mammalian cells in order to maximum expression in that mammalian cells for the efficient and stable enzyme activity in terms of luminescence to be used in medical research and diagnostics applications." While applicant's patent application states that the foregoing is desirable,[3] none of Szittner et al., Mao et al., Zolotukhin et al., Greer et al., or Lowe et al. teach that it would be desirable to stabilize or increase the expression of a bacterial LuxA protein by codon optimization. Szittner et al. and the Mao et al. abstract describe bacterial Lux systems, but do not mention codon optimization or that it would be desirable to codon optimize a bacterial luciferase gene. Zolotukhin et al., Greer et al., and Lowe et al. describe codon optimization for jellyfish GFP and firefly luciferase, but do not state that it would be desirable to codon optimize a bacterial luciferase gene.[4] Because the pertinent cited art fails to provide any motivation or suggestion to make a codon-optimized

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to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991)."

[3] In re Fine, 837 F.2d 1071, 1075 (Fed. Cir. 1988) ("One cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention"); *ACS Hosp. Sys., Inc. v. Montefiore Hosp.*, 732 F.2d 1572, 221 USPQ 929 (Fed. Cir. 1984); *In re Dow Chem. Co.*, 837 F.2d 469, 473, 5 USPQ2d 1529, 1531 (Fed. Cir. 1988) (both the suggestion and reasonable expectation of success must be found in the prior art, and not in the applicant's disclosure).

[4] The jellyfish *gfp* and firefly luciferase systems differ markedly from the bacterial luciferase system of the present invention. For example, GFP is a protein that itself emits fluorescence without requiring cofactors or substrates. Bacterial luciferase, in comparison, catalyzes a reaction that involves the oxidation of a reduced riboflavin phosphate and a long-chain fatty aldehyde substrate, the reaction involving luciferase binding to the substrate. As another example, both jellyfish GFP and firefly luciferase are monomeric proteins, whereas bacterial luciferase is a heterodimer composed of two proteins (LuxA and LuxB).

bacterial *luxA*, the examiner's burden of establishing a *prima facie* case of obviousness has not been met. Accordingly, withdrawal of these rejections is respectfully requested.

Conclusion

The currently pending claims before the examiner are supported throughout the specification and are patentable over the prior art. No new matter has been added. This application is now in full condition for allowance, and such action is respectfully requested.

The Commissioner is hereby authorized to charge any underpayment or credit any overpayment of fees under 37 CFR 1.16 or 1.17 as required by this paper to Deposit Account 50-3110.

The examiner is cordially invited to call the undersigned if clarification is needed on any matter within this response, or if the examiner believes a telephone interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,

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